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Rapid Aquatic Toxicity Assay Using Incorporation of Tritiated-Thymidine into Sea Urchin, 'Arbacia punctulata', Embryo Evaluation of Toxicant Exposure Procedures

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16. ABSTRACT

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This paper presents a comparison of toxicant exposure procedures using the Arbacia embryo thymidine incorporation test. Toxicant exposure began before, at the time of, or after fertilization and continued for 4 h following fertilization. In addition to the eight organic chemicals tested for comparison to acute toxicity values for other species, several chemicals with embryotoxic potentials (tumor promoters and teratogens) were tested to determine differential sensitivities of exposed life-stages: unfertilized egg, fertilization, and early embryo. EC 50 values for any one substance were not significantly changed by exposure modification. Toxicity values for exposures that included fertilization as well as early embryo growth were at least as sensitive as post-fertilization exposure values for all compounds tested except one. Because of technical ease and potential sensitivity, toxicant exposure that includes fertilization as well as early embryo growth (but not unfertilized egg exposure) is recommended for future testing.

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Rapid Aquatic Toxicity Assay Using Incorporation of Tritiated-Thymidine into Sea Urchin, *Arbacia punctulata*, Embryo: Evaluation of Toxicant Exposure Procedures

REFERENCE: Nacci, D. E. and Jackim, E., **"Rapid Aquatic Toxicity Assay Using Incorporation of Tritiated-Thymidine into Sea Urchin, *Arbacia punctulata*, Embryo: Evaluation of Toxicant Exposure Procedures,"** *Aquatic Toxicology and Hazard Assessment: Eighth Symposium, ASTM STP 891*, R. C. Bahner and D. J. Hansen, Eds., American Society for Testing and Materials, Philadelphia, 1985, pp. 382-394.

ABSTRACT: Toxicity of substances in seawater was measured using growth inhibition of embryonic sea urchins during a short period after fertilization. Growth of *Arbacia punctulata* embryos was monitored by incorporation of tritium-labeled thymidine, a nucleoside incorporated into newly synthesized deoxyribonucleic acid (DNA). Sensitivity and ranking ability of this rapid *Arbacia* embryo test were judged by comparison of toxicity values for several organic industrial chemicals using this test and two standard acute aquatic toxicity tests. Median effective toxicity values (EC_{50} values) for early embryos compared favorably to median lethal values (LC_{50} values) obtained in standard acute toxicity tests using *Pimephales promelas* (fathead minnow) and *Daphnia magna* (water flea).

This paper presents a comparison of toxicant exposure procedures using the *Arbacia* embryo thymidine incorporation test. Toxicant exposure began before, at the time of, or after fertilization and continued for 4 h following fertilization. In addition to the eight organic chemicals tested for comparison to acute toxicity values for other species, several chemicals with embryotoxic potentials (tumor promoters and teratogens) were tested to determine differential sensitivities of exposed life-stages: unfertilized egg, fertilization, and early embryo. EC_{50} values for any one substance were not significantly changed by exposure modification. Toxicity values for exposures that included fertilization as well as early embryo growth were at least as sensitive as post-fertilization exposure values for all compounds tested except one. Because of technical ease and potential sensitivity, toxicant exposure that includes fertilization as well as early embryo growth (but not unfertilized egg exposure) is recommended for future testing.

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Additional development, refinement, and standardization of this fertilization and early embryo test should further increase its utility in toxicity testing. Because this test requires only 4 h to exposure and can be correlated with standard measurements of toxicity, it could be used in early stage hazard evaluation in conjunction with other aquatic bioassays.

KEY WORDS: toxicity, seawater, sea urchins, aquatic toxicity, marine embryo, tritiated-thymidine incorporation, *Arbacia punctulata*

Standard aquatic toxicity tests using macroinvertebrates and fishes have been used to assess acute toxicity but are cumbersome for screening large numbers of substances. Rapid, small volume toxicity tests, such as those employing larval or embryo stages, offer some practical advantages for generating toxicity rankings and are useful when they can be correlated with accepted measurements of toxicity.

The sea urchin is one of the most well-studied of all biological systems. A wealth of information has been accumulated on the embryological, cellular, and biochemical events associated with fertilization and early development [*1*]. The field of aquatic toxicology has taken advantage of this system not only because of this sound scientific foundation but because of some practical advantages associated with its use: ease of handling—adults and gametes, ubiquitous domain, and sensitivity as surrogate species [*1*]. A broad range of pure compounds have been tested using sea urchin embryo systems. The classic work of Hagstrom and Lönning [*2*] documents teratogenic effects of certain drugs, including thalidomide. Other research has included drugs [*3*], metals [*4–6*], neuropharmalogical agents [*7*], pesticides [*8*], and organics, such as oil dispersants and polychlorinated biphenyls [*8–11*]. Complex environmental mixtures have also been tested using various species of sea urchins. Several Japanese laboratories, particularly that of Kobayashi, have accumulated extensive documentation through detailed observation on the response of sea urchin gametes and various larval stages to polluted waters and effluents [*12–15*]. Municipal wastewaters of California were monitored by Oshida et al [*16*] using a standardized 48-h lethality bioassay for sea urchin larvae.

Several novel approaches using sea urchin systems describe the application of relatively rapid, quantitative endpoints correlated with biologically significant events. Bay et al [*17*] suggest that the measurement of a pigment (echinochrome) produced by an appropriately developed advanced larval stage be used to estimate toxicity in exposed versus control embryos. Dinnel et al [*8,18,19*] have quantitated toxicity to sperm by reduced fertilization success in briefly exposed gametes.

Other novel procedures using sea urchin systems have been described, which define toxicity as disruption of fundamental developmental processes, that is, those associated with DNA synthesis. Hose and Puffer [*20*] have quantitated chromosomal and cytogenetic anomalies in embryos and gametes of exposed adults and directly exposed gametes. Pagano [*21*] has used autora-

diographic techniques to quantitate mitotic indices, as Karnovsky et al had for an extensive series of organic compounds using another echinoderm, the sand dollar [*22*]. The effects of toxicants on development has generally been by observational procedures, informational but tedious and often subjective and, therefore, unsuitable for screening purposes. Very early embryo growth has not been quantitatively evaluated.

The procedure described here incorporates aspects of both approaches described above. The early embryo growth test measures toxicity to briefly exposed gametes and embryos as the inhibition of DNA synthesis in growing embryos. The endpoint used, liquid scintillation counted incorporated tritiated-thymidine ([^{3}H]-thymidine), is a standard quantitative technique to rapidly estimate net toxicity to thousands of organisms per treatment.

This paper describes results of sea urchin toxicity tests using incorporation of [^{3}H]-thymidine to quantitate impacts on growth of embryos during the first four hours of development after various regimes of toxicant exposure. Incorporation of [^{3}H]-thymidine is known to be stimulated by fertilization [*23*] and has been shown to be proportional to DNA synthesis and cell division in the growing embryo [*24*]. Toxicity is demonstrated by reduced incorporation, inversely proportional to concentration of toxicant. Initial findings indicated that this test is more efficient than the development test and is comparable in sensitivity and toxicity values [*25*]. Work has continued to further characterize this system and investigate potentially useful modifications.

The assay, as originally developed, employed early embryo exposure and incorporation periods. The present study explores the effects of toxicant exposure to the egg, before, during, and after fertilization, and was undertaken to determine (1) if the sensitivity of the test increases by including additional life stages and (2) if sensitivity unique to life-stage(s) is demonstrated with teratogenic or other classes of compounds. Additionally, results for compounds tested over several seasons were used to evaluate response to toxicants using gametes obtained from animals kept under laboratory conditions for over one year.

Methods and Materials

General Method

Sea urchin gametes or fertilized eggs were added to small volumes of seawater containing varying concentrations of toxicant. For pre-fertilization exposure, eggs were added to toxicant 1 h before addition of sperm. At-fertilization exposure required simultaneous addition of eggs and sperm to toxicant. Post-fertilization exposure employed eggs fertilized in uncontaminated seawater, with toxicant added at 1 h after fertilization. In all cases, exposure continued during early embryo growth. At 2 h after fertilization, [^{3}H]-thymidine was added directly to exposure vials. At the end of a 2-h incorporation

period, embryos were collected and washed on filters. Incorporation of [^{3}H]-thymidine was determined by liquid scintillation counting. Linear regression analysis was employed to generate EC_{50} values and 95% confidence intervals.

Test Animals

The sea urchins, *Arbacia punctulata*, were obtained commercially from biological supply companies in Panacea, FL, and Woods Hole, MA. Males and females were held in separate tanks in continuously flowing filtered seawater (15 to 20°C, 30 ± 2 parts per hundred salinity) and fed *Luminaria* (kelp), ad libitum. These holding conditions produced continuously fertile animals.

Seawater

Seawater from Narrangansett Bay, RI, was collected, filtered through particle cartridge filters, and autoclaved for 15 min at 110°C, before storage. Quantities sufficient for several weeks were processed at one time. Immediately before use, seawater was filtered through a Whatman No. 1 prefilter and a Millipore 0.22 μ-filter, in series. This procedure provided water of consistent quality that was sufficiently low in particles and bacteria for daily use in the thymidine incorporation assay.

Spawning and Gamete Quantification

The test animals were spawned separately using electrical stimulation. Eggs were collected in filtered seawater and washed twice before use [22]. All subsequent procedures were conducted at 20°C.

Washed eggs were diluted in filtered seawater, subsampled, and counted in a Sedgwick-Rafter counting chamber using a dissecting microscope. Egg suspensions were adjusted to approximately 5000 eggs/mL (fifty-fold final concentration). Sperm were collected by syringe and diluted ten-fold (by volume) with filtered seawater, immediately before use. One millilitre of egg suspension (5000 eggs) was dispensed into culture dishes containing 49-mL filtered seawater (final egg concentration, 100/mL). At the time of fertilization, 0.1-mL diluted sperm was added to each dish. Sperm-egg ratios were between 100 and 1000 to 1, producing maximal control fertilization.

Exposure and Fertilization

Each experiment simultaneously tested a single compound over a range of concentrations using three exposure procedures. In each case, the test compound was dissolved in seawater (with solvent when necessary) and added with gentle mixing by pipet to egg suspensions. Test compounds were added to culture dishes at one of three times: 1 h before fertilization, immediately

(less than 5 min) before fertilization, or 1 h after fertilization. These exposure conditions are referred to as pre-, at-, and post-fertilization, respectively.

Eggs in culture dishes remained undisturbed for 1 h after fertilization. At that time, toxicant was added to certain appropriate dishes (post-fertilization exposure samples), and all dishes were sampled to monitor fertilization. Fertilization was observed microscopically by the appearance of fertilization membranes. Tests in which control egg batches were less than 90% fertilized were discarded [*1*].

Toxicants

Eight compounds used were selected because of their varied solubilities and toxicities from among those tested in the structure-activity program at the U.S. Environmental Protection Agency, ERL—Duluth [*26*]. Chemical characterizations of these industrial chemicals are available [*26*]. They were 2-(2-ethoxyethoxy)-ethanol, 2-methyl-2,4-pentanediol, 2-methyl-1-propanol, 2,2,2-trichloroethanol, 2,4-pentanedione, 2-chloroethanol, hexachloroethane, and pentachlorophenol. These compounds were initially tested with this thymidine incorporation assay using the post-fertilization exposure method in early 1983 [*25*]. They were retested using pre-, at-, and post-fertilization exposures for this study. In addition, a few compounds were selected for their potential as teratogens or fertilization-inhibiting agents, for example, copper [*27*]. These compounds were copper (as copper sulfate), 4β-phorbol 12-myrisate 13-acetate, phorbol-12,13-dibutyrate, 4-β-phorbol, phorbol-12,13-diacetate, and di(2-ethyl-hexyl)phthalate.

All compounds were initially dissolved in filtered seawater, distilled water, or ethanol such that the concentration of solvent did not exceed 0.1%, which was demonstrated to be nontoxic in control embryos (unpublished data). Seawater exposure solutions were not diluted by more than 2% upon addition of toxicant. Solutions were made just before testing and diluted to (nominal) final concentrations as described. Exposure concentrations were not measured by chemical analysis.

Thymidine Incorporation Assay

Two hours after fertilization, embryo suspensions were subdivided and tritiated-thymidine added. Ten millilitres of the embryo suspension (1000 embryos) from each culture dish were added to 20-mL glass vials containing 1.52 μCi of tritiated-thymidine (20 Ci/mmol) in 0.1-mL seawater. Final activity was 0.138 μCi/mL. After 2 h of thymidine incorporation, the embryo suspension was filtered onto paper filters using three, 12-filter manifolds. Paper filters were cut from commercially available paper coffee filters. Embryos were washed on the filters sequentially with 5-mL seawater, 5% trichloroacetic acid, 95% ethanol, and 1-mL deionized water. Filters were then placed

in scintillation vials and treated with 0.1-mL tissue solubilizer (Soluene-350, Packard Instruments, Inc.) for 30 min. Chemiluminescence quenching scintillation cocktail (Dimilume-30, Packard Instruments, Inc.) was added and disintegrations per minute (dpm) were obtained to determine the degree of thymidine incorporation by the developing embryos.

Experimental Design and Data Analysis

Preliminary tests were used to determine test concentrations approximately equal to EC_{20}, EC_{40}, EC_{60}, EC_{80}, and EC_{100} for each compound. Six treatments, control and these five concentrations, were tested in duplicate for each exposure routine (18 treatments per experiment). For a typical experiment, using this design, a 55% difference from the mean incorporated counts was statistically significant ($\alpha = 0.05$). The coefficient of variation of radioactive counts (calculated as variance divided by mean counts) was 19.0%.

Data on the incorporation of [^{3}H]-thymidine were plotted against the log of the nominal concentration of the test compound. Linear regression analyses were performed on each experiment, categorized by method of exposure. Within experiments, effects of various exposure condition were compared by analysis of covariance to test equality of slopes and intercepts of calculated lines. Effective concentrations which induced 50% inhibition of control incorporation values, EC_{50}s, and 95% confidence intervals were calculated [*28*]. Significant differences between EC_{50} values were determined by the method of Finney [*29*].

Results

Toxicity

Eleven compounds were tested in experiments using three methods of exposure simultaneously. These results are presented in Table 1 as EC_{50} values with 95% confidence limits. The compounds are ranked from least toxic to most toxic. Although methods varied in exposure duration and life stage, when EC_{50} values were compared statistically, there were no significant differences between the three methods of exposure ($p < 0.05$). For each compound, statistical comparisons were insensitive because differences between treatments were small relative to variability (as evidenced by confidence intervals). Qualitatively, methods were compared according to frequency of most sensitive response for all compounds tested. Using this comparison, at- or pre-fertilization exposure methods were equally sensitive. Taken together, these two methods yielded most sensitive results for ten of eleven compounds.

Three compounds, in addition to those listed in Table 1, were nontoxic within the range of tested concentrations. Phorbol-12,13-diacetate, and 4-β-

TABLE 1—*Median effective concentrations (and 95% confidence intervals) as determined by the inhibition of tritiated-thymidine using* Arbacia *embryos. Experiments were conducted with exposures beginning 1 h before fertilization (pre), or at the time of fertilization (at), or 1 h after fertilization (post). All exposures continued through the period of thymidine incorporation (4 h after fertilization). EC_{50} values and 95% confidence intervals for compounds tested in the sea urchin embryo incorporation test.*

Compound	Exposure	EC_{50} mg/L	95% Confidence Interval	
			Lower Limit, mg/L	Upper Limit, mg/L
2-(2-Ethoxyethoxy)-Ethanol	pre	10 660	5 576	20 893
	at	12 421	7 290	22 470
	post	12 694	8 483	19 707
2-Methyl-2,4-Pentanediol	pre	8 109	6 368	10 280
	at	8 742	6 086	12 938
	post	10 248	8 042	13 128
2-Methyl-1-Propanol	pre	2 022	1 357	2 702
	at	1 876	1 097	2 783
	post	2 185	1 753	2 901
2-Chloroethanol	pre	596	219	1 211
	at	931	531	1 601
	post	1 062	628	1 822
2,2,2-Trichloroethanol	pre	445	306	592
	at	446	230	687
	post	293	81	471
2,4-Pentanedione	pre	105.4	56.4	170.2
	at	60.8	4.4	160.3
	post	395.0	222.3	886.4
Hexachloroethane	pre	6.05	4.67	7.60
	at	4.97	4.04	5.91
	post	8.31	5.80	12.93
Pentachlorophenol	pre	0.33	0.22	0.47
	at	0.35	0.26	0.47
	post	0.47	0.20	1.24
4β-Phorbol 12-Myristate 13-Acetate, μg/L	pre	0.84	0.16	4.63
	at	1.25	0.63	2.50
	post	2.47	1.08	6.49
Phorbol-12,13-Dibutyrate	pre	11.4	0.1	91.6
	at	25.3	16.4	39.6
	post	46.0	18.0	175.7
Copper	pre	14.0	5.6	29.5
	at	7.9	2.2	18.0
	post	90.6	23.4	910.6

phorbol were tested at a maximum concentration of 1 mg/L. The highest concentration of di(2-ethyl-hexyl)phthalate tested was 100 mg/L.

Fertilization Inhibition

The relationship between the inhibition of fertilization and the inhibition of [^{3}H]-thymidine incorporation for all compounds (pooling data), examined by

regression analysis, revealed no significant correlation. When data are categorized by compound, and subjected to linear regression analysis (not shown), only one compound, 2-chloroethanol, demonstrated a significant correlation ($p < 0.05$) between incorporation and fertilization. Fertilization inhibition was seen only at concentrations of toxicant which resulted in extremely reduced incorporation of tritiated-thymidine ($> EC_{50}$ levels) and is, therefore, not as sensitive a measurement of toxicity.

Test Comparisons

Figures 1 and 2 compare toxicity results for eight compounds derived by the at-fertilization exposure for the sea urchin embryo (EC_{50} values) versus the LC_{50} values from standard aquatic assays for *Pimephales promelas* (fathead minnow) and *Daphnia magna* (water flea). Data were provided by Dr. R. V. Thurston, Montana State University Fisheries Bioassay Laboratory, Bozeman, MT (manuscript in preparation) for the 96-h flow-through test for *Pimephales* and the 48-h static-renewal test for *Daphnia*. An analysis of covariance of these linear regressions, reveals that for these compounds, results from *Arbacia* and *Pimephales'* tests are statistically equivalent. The *Daphnia* test is slightly more sensitive than but highly correlated with the *Arbacia* test (equality of slopes).

EC_{50} values determined for the current study or the previous study from this laboratory [*22*] using the sea urchin embryo thymidine-incorporation test are compared in Table 2. There are no significant differences between values for any compound.

Discussion

Fertilization Exposure

Exposure of the egg for up to 1 h before fertilization did not significantly change toxicity values measured by inhibition of tritiated-thymidine incorporation nor seriously impede fertilization. Fertilization inhibition was not highly correlated with this measured toxicity and was generally seen only at high test concentrations ($> EC_{50}$ levels), if at all. Dinnel et al demonstrated that pre-exposing sperm and minimizing sperm to egg ratio makes fertilization a critical endpoint in toxicity evaluation [*8*]. A logical extension of this study includes a comparison of sperm and embryo sensitivities.

By experimental design, the exposure regime that includes the fertilization period subjects embryos to a longer period in toxicant than post-fertilization exposure. This procedure is expected to yield more sensitive results without increasing the length and complexity of the assay. At-fertilization exposure is, therefore, recommended for previously untested compounds.

Of the three most toxic compounds tested, two, 4-β-phorbol 12-myristate

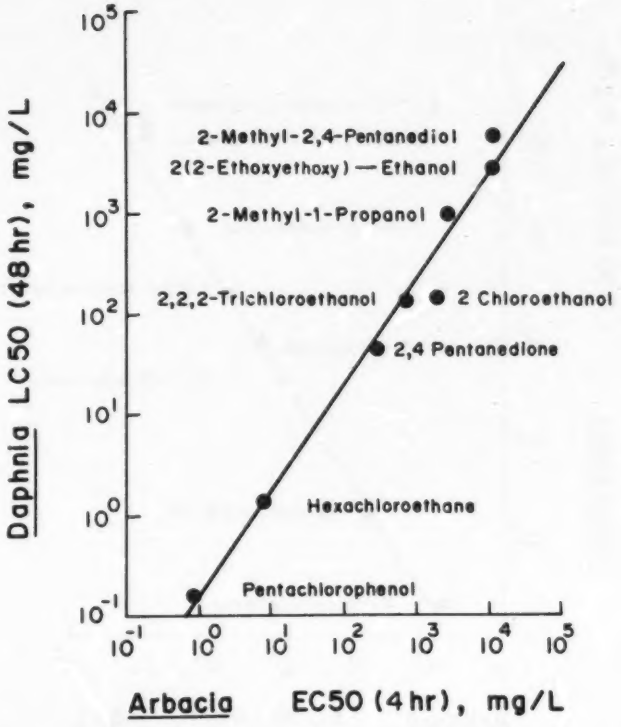

FIG. 1—*Toxicity values (EC_{50}, mg/L) as determined by the at-fertilization exposure method using tritiated-thymidine incorporation are compared with values (LC_{50}, mg/L) for* Daphnia magna *when tested in standard acute tests. (Data supplied by Dr. R. V. Thurston, Montana State University Fisheries Bioassay Laboratory, Bozeman, MT, manuscript in preparation.) The equation for the line is*

$$y = 1.018(x) + (-0.4184),\ r^2 = 0.9909$$

where x = *at-fertilization exposure, EC_{50}.*

13-acetate and phorbol-12,13-dibutyrate, are embryotoxicants, described as teratogens or tumor-promoters in some systems [*30*], and copper induces severe cleavage anomalies associated with polyspermy in the sea urchin [*5*]. The compound, 4-β-phorbol 12-myristate 13-acetate, one of the most potent tumor-promoters known, has also been shown to have deleterious effects on sea urchin development at very low concentrations by other authors [*31*]. Inter-

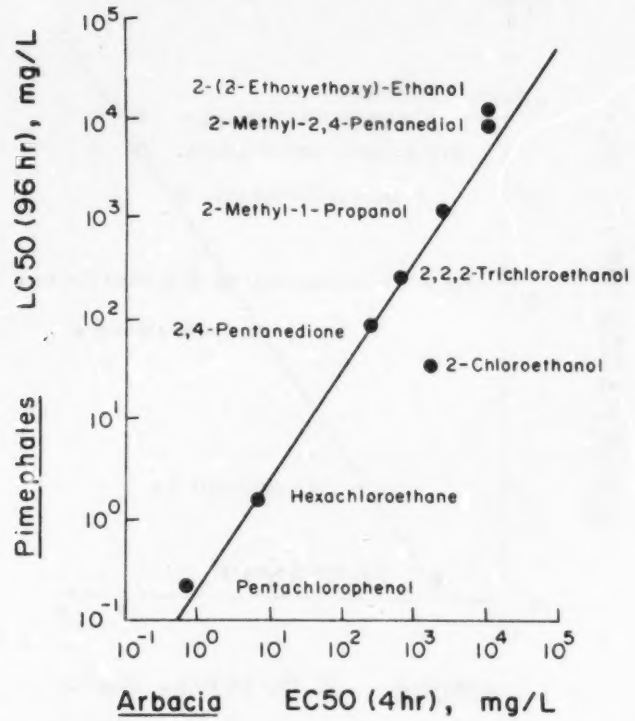

FIG. 2—*Toxicity values (EC_{50}, mg/L) as determined by the at-fertilization exposure method using tritiated-thymidine incorporation are compared with values (LC_{50}, mg/L) for* Pimephales promelas *(fathead minnow) when tested in standard acute tests. (Data supplied by Dr. R. V. Thurston, Montana State University Fisheries Bioassay Laboratory, Bozeman, MT, manuscript in preparation.) The equation for the line is*

$$y = 1.0019(x) + (-0.28682), r^2 = 0.9292$$

where x = *at-fertilization exposure, EC_{50}.*

estingly, tumor-promoters while characterized by their ability to stimulate growth in "initiated" cells, are inhibitory to embryonic cells. These tumor-promoters have demonstrated similar relative toxicities to sea urchin embryo and cultured mouse embryo. The compounds, 4-β phorbol and phorbol 12, 13 diacetate, weaker promoters in the mouse system [*30*], were nontoxic up to 1 mg/L to the sea urchin embryo. Di(2-ethyl-hexyl)phthalate has also been

TABLE 2—*Comparison of toxicity values as determined by the inhibition of tritiated-thymidine incorporation using* Arbacia *embryos by the authors for this study and as published* [25]. *Both series of experiments were conducted with exposures beginning 1 h after fertilization and continuing through the period of incorporation (4 h after fertilization).* · *Comparison of toxicity values determined at different times using tritiated-thymidine incorporation of* Arbacia *embryos exposed to toxicant after fertilization.*

	Present Study		Jackim and Nacci, [25]	
Compound	EC_{50}	95% Confidence Interval, mg/L	EC_{50}	95% Confidence Interval, mg/L
2-(2-Ethoxyethoxy)-Ethanol	12 694	8 483 to 19 707	11 720	8 656 to 15 967
			10 868	7 704 to 15 332
2-Methyl-2,4-Pentanediol	10 248	8 042 to 13 128	13 555	9 757 to 18 832
			9 486	7 266 to 12 385
2-Methyl-1-Propanol	2 185	1 753 to 2 901	2 601	1 859 to 3 646
			2 840	1 558 to 5 183
2-Chloroethanol	1 062	628 to 1 822	1 412	862 to 2 309
			1 834	1 654 to 2 035
2,2,2-Trichloroethanol	293	81 to 471	496	296 to 833
			625	474 to 821
2,4-Pentanedione	395	222 to 886	169	81 to 240
			206	68 to 561
Hexachloroethane	8.31	5.80 to 12.93	8.51	7.43 to 9.19
			9.32	8.29 to 10.60
Pentachlorophenol	0.47	0.20 to 1.24	0.87	0.23 to 3.52
			0.71	0.34 to 1.64

implicated as a tumor promoter and a teratogen in some aquatic species [*32*] but was nontoxic when tested up to its solubility limit in seawater. Similarities between tumor-promoters, teratogens, and embryotoxicants are not established but it is shown here that the sea urchin gamete-embryo system is sensitive to certain agents with these classifications in the microgram per litre (μg/L) range. No unique life-stage sensitivities were observed.

Test Correlations

Correlations between results of the present study and a previously published study [*25*] using the sea urchin embryo thymidine incorporation test support the use of gametes from laboratory-held adult sea urchins for testing purposes. There were no seasonal changes in sensitivity observed. Additionally, for these eight selected compounds, the *Arbacia* embryo thymidine incorporation assay and two standard acute aquatic tests using *Daphnia* and *Pimephales* rank toxicants similarly. The single anomalous compound in the latter comparison is 2-chloroethanol. This compound is metabolized by some organisms (for example, fathead minnow) to a more toxic compound [*26*]. Toxicity of the compound in the sea urchin embryo and egg suggests that it is not similarly transformed there. This toxicity agrees well with that predicted for the unchanged compound, based on structure-activity relationships [*26*].

It is surprising that the toxicities to marine and freshwater organisms of the organic industrial test compounds were so similar. Such similarities for compounds (such as metals), which ionize and form complexes differently in salt and freshwater, would probably not exist.

Conclusions

To predict general toxicity, nonlethal effects must be correlated with basic and fundamental life processes. DNA synthesis is essential to growth in all living systems. Quantitation of newly synthesized DNA using [^{3}H]-thymidine is a well-characterized and often employed research method. It is rapid, technically simple, and sensitive. Its role in toxicity testing is novel. Embryonic development, characterized by rapid growth and coordinately high rate of DNA synthesis, is an ideal system to quantitate growth inhibition as measured by reduced [^{3}H]-thymidine incorporation. Additional methods development would increase the utility of this test. Modifications are necessary to reduce variability within treatments seen using the present processing techniques. Alternatively, a sufficiently sensitive, nonradioactive method could be used to quantitate increasing DNA content in growing embryos.

Results suggest that the early embryo growth test described here could be used for screening toxic substances efficiently.

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